Sex-Dependent Metabolic, Neuroendocrine, and Cognitive Responses to Dietary Energy Restriction and Excess


Introduction

Regulation of Energy Balance

To ensure the presence of sufficient energy stores to endure periods of food scarcity and avoid obesity

Eating disorders

- Excess food intake
  - Obesity
  - ↑ Risk of
    - Cardiovascular disease
    - Type 2 diabetes
    - Premature death

- Restricted food intake
  - Anorexia nervosa

Eating Disorders

Excess food intake
- Obesity
- ↑ Risk of
  - Cardiovascular disease
  - Type 2 diabetes
  - Premature death

In laboratory animals
- Rats & mice typically overate
- Increase disease incidence & shortened life span
- Comparison to caloric restriction/ intermittent fasting gr.

Overeating increase the ability of animals
- to survive periods of famine
- to reproduce
- to provide nourishment to offspring

Eating Disorders

Restricted food intake

Anorexia nervosa (AN)
- an often fatal eating disorder with unclear biological basis
- the affected individual severely restricts food intake & increases energy expenditure despite progressive emaciation
- AN is classified as an obsessive-compulsive psychiatric disorder in human

Gull W (1874), Chan J et al. (2003), Yager J et al. (2005), Humphries L et al. (2003).

Sex-dependent Differences in Response to Energy Intake

- Severe CR inhibits the reproductive cycle in female rodents without adversely affecting in males
- Women with AN typically exhibit amenorrhea
- The influence of energy intake on disease susceptibility may also differ in men and women
  Maxwell S (1998)

Involvement of the Brain in the Regulation of Energy Balance

Hypothalamic neurons & other brain neurons control appetite
- In human
  Performance on cognitive tasks in obese subjects < controls
- Learning and memory ability in AN patients ≥ controls
  Strupp B et al. (1996), Connan F et al. (2006).
- In animals
  Excessive energy intake has harmful effect on cognitive function
  Winocur G et al. (2005).

Aims of the study

Analyze the behavioral, neurochemical and endocrine responses of male & female rats to high, medium and very low energy diets

1. Mechanisms by which the brain responds to change in energy intake
2. How those responses are translated into behavioral, neurochemical and endocrine systems
3. Different responses between sexes

Methods to Test Effect of Energy Intake and Sex-dependent Responses on

- Body weight & organ weight
- Ambulatory activity
- Reproductive physiology
- Plasma lipid
- Energy metabolism
- Growth hormone
- Cognitive performance
- Neurotransmitters and metabolites of brain
- BDNF levels
Data analysis

- Mean ± SEM
- ANOVA
- Student’s t-test
- P value < 0.05

Method

Animals

Sprague Dawley rats
- 47 ♀ & 47 ♂
- singly housed
- 12 h light/dark cycle (light on at 7:00)

Rats were divided in 5 gr. on different diets

Ambulatory activity assessment

Training using straight runway & 14-unit T-maze (3 days)

Testing 14-unit T-maze again

Collecting blood sample and obtaining tissues

Methods to Test Effect of Energy Intake and Sex-dependent Responses on

- Body weight & organ weight

Exp 1  Testing Effects of Dietary Energy Intake on BW and Organs’ Weight

- Body weight measurement
- Recording food consumption
- Calculating caloric intake
- Measuring the wet weights of organs at necropsy & normalized organ weight to terminal BW of rats in the same diet group
Tissue collection

- Tissues were flash frozen on dry ice at -80°C, kept until analysis.
- Isoflurane anesthesia
- Decapitation
- Dissected to obtain hippocampus, striatum, cortex, cerebellum, brainstem, heart, liver, kidneys, adrenal glands, gonads, pancreas, spleen.

Result

Caloric restriction

- BW

Caloric excess

- BW in males > females

CR & IF

- Adrenal size in females > males
- Gonadal size in females

Table 1: Effects of dietary energy intake on body weight and organ weights in adult rats (n=10 per group).

<table>
<thead>
<tr>
<th>Group</th>
<th>Weeks</th>
<th>Brainstem</th>
<th>Heart</th>
<th>Liver</th>
<th>Kidney</th>
<th>Adrenal (L)</th>
<th>Kidney (L)</th>
<th>Gonad (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>0.05 ± 0.02</td>
<td>0.07 ± 0.02</td>
<td>0.05 ± 0.02</td>
<td>0.07 ± 0.02</td>
<td>0.05 ± 0.02</td>
<td>0.07 ± 0.02</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td>25% CR</td>
<td>12</td>
<td>0.04 ± 0.02</td>
<td>0.06 ± 0.02</td>
<td>0.04 ± 0.02</td>
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<td>0.06 ± 0.02</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>40% CR</td>
<td>12</td>
<td>0.03 ± 0.02</td>
<td>0.05 ± 0.02</td>
<td>0.03 ± 0.02</td>
<td>0.05 ± 0.02</td>
<td>0.03 ± 0.02</td>
<td>0.05 ± 0.02</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>50% CR</td>
<td>12</td>
<td>0.02 ± 0.02</td>
<td>0.04 ± 0.02</td>
<td>0.02 ± 0.02</td>
<td>0.04 ± 0.02</td>
<td>0.02 ± 0.02</td>
<td>0.04 ± 0.02</td>
<td>0.02 ± 0.02</td>
</tr>
</tbody>
</table>

Note: Data are presented as mean ± SEM. *P < 0.05 compared to control group.

Exp 2: Testing Sex-Related Effects of Dietary Energy Restriction on Spontaneous Activity Levels in Rats

- Ambulatory activity assessment
  - Digiscan open-field activity monitor
  - Recorded at 7h -10 h and 19h -22h
  - Multiple observers
    - Observed & scored at random times during the day

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Females consumed less food & calories than males
- Caloric intake was stable during the experiment

Week 2

- Body weight & organ weight
- Ambulatory activity
- Reproductive physiology
- Energy metabolism
- Growth hormone
- Cognitive performance
- Plasma lipid
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- BDNF levels

Week 21

- Body weight & organ weight
- Ambulatory activity
- Reproductive physiology
- Energy metabolism
- Growth hormone
- Cognitive performance
- Plasma lipid
- Neurotransmitter and metabolites of brain
- BDNF levels

Week 22

- Body weight & organ weight
- Ambulatory activity
- Reproductive physiology
- Energy metabolism
- Growth hormone
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- Plasma lipid
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- BDNF levels

Week 23

- Body weight & organ weight
- Ambulatory activity
- Reproductive physiology
- Energy metabolism
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Exp 3  Evaluation of Reproductive Cycle in Female Rats and Relevant Hormones in both Sexes

Vaginal smear test
- Monitoring daily uterine activity
- Scored as regular, irregular or absent cyclicity
  - Regular estrous cycle
    - typical 4-d cycle: d1-diestrous, d2-proestrous, d3-estrous, d4-metestrous
  - Irregular estrous cycle
    - represent cycles less than 4 days in the pattern
  - Absent estrous cycle
    - no regularity during their typical 4-d cycle

Plasma hormone analysis

<table>
<thead>
<tr>
<th>Testosterone</th>
<th>Corticosterone</th>
<th>Estradiol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measured by RIA</td>
<td></td>
<td>Measured by RIA</td>
</tr>
</tbody>
</table>

Blood sample collection

- Overnight fast
- Blood samples were collected
- EDTA-heparinized centrifuge tube
- Plasma was aspirated, stored at -20°C until analysis
- Centrifuged at 3000 rpm, 30 min at 4°C

Dietary Restriction and Excess Disturb Estrous Cycles

Hyperactivation of Adrenal Stress Response to Reduced Energy Availability in Females

Hyperactivation of Adrenal Stress Response to Reduced Energy Availability in Females

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Methods to Test Effect of Energy Intake and Sex-dependent Responses on

1. Body weight & organ weight
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3. Reproductive physiology
4. Plasma lipid

Methods to Test Effect of Energy Intake and Sex-dependent Responses on

1. Body weight & organ weight
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4. Plasma lipid
5. Energy metabolism
6. Growth hormone
7. Cognitive performance
8. Neurotransmitter and metabolites of brain
9. BDNF levels

Plasma lipid analysis

Plasma samples analyzed for:
- Triglyceride
- Cholesterol
- LDL
- HDL
- 3-hydroxybutyrate

Using Automated COBAS centrifugal analyzer

Dietary Energy Restriction Improved Atherogenic Profile in Both Sexes

Cholesterol
LDLC
3-HB

Control
20% CR
40% CR
IF
HFG

* p < 0.05
** p < 0.01
*** p < 0.001

Plasma Insulin Tended to Decrease on CR Diet

While Plasma Glucose Was Not Effected

Plasma hormones and glucose analysis

Measured by RIA
Ghrelin
Adiponectin
Leptin
Insulin

Measured by ELISA

Glucose levels were measured by glucometer

Exp 4 Evaluation of Sex-Dependent Responses of Plasma Lipids to Altered Energy Intake

Exp 5 Effects of Altered Dietary Energy Intake on Energy-Regulating Hormones in Both Sexes
Exp 6  Sex-dependent Response of GH and Prolactin to Altered Dietary Energy Intake

GH  Prolactin were measured by RIA

Dietary Restriction induced different response in both sexes

Exp 7  Elucidation of Sex-dependent Effects of Altered Dietary Energy Intake on Cognition

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**Pre-maze Testing: Straight Runway**

- Each trial: rat had to move to goal area within 10 sec
- Each trial was done every 2 min
- 3 failures to escape within 60 sec → be removed from the experiment

**14-unit T-maze**

**Acquisition Trial**

- Shock Frequency & Time in Female Rats on 40% CR

**Retention Trial**

- Shock Frequency in Female Rats on 40% CR
Differences in Diet & Sex Did Not Affect Retention Error and Time

These results show that females respond to CR with a heightened state of cognitive ability, compared with males.

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Exp 6 Determination Whether the Sex Differences in Physiological and Behavioral Responses to Altered Dietary Energy Intake Are Associated with Differential Changes in Neurotransmitters

Tissue neurotransmitters & protein analyses

- Norepinephrine (NE)
- Dopamine (DA)
- Dihydroxyphenylacetic acid (DOPAC)
- 5-hydroxy indoleacetic acid (5HIAA)
- Serotonin (5HT)
- Homovanillic acid (HVA)

HPLC Analysis of Neurotransmitters

- Measured by HPLC with electrochemical detection
- Calculated as pg/mg of tissue weight
These results show that dietary energy intake was associated with differential changes in neurotransmitters in various brain regions (hippocampus, cerebral cortex, cerebellum and striatum), particularly DA levels in hippocampus.
Levels of BDNF in plasma, hippocampus, cerebral cortex measured by ELISA

This study

- Strong sex-based difference in the animals' response to dietary disruption
- Physiological adjustment to the alteration of diet in males < females rats
- Females --> significant whole-body endocrinological realignment, especially in mild & severe CR

These findings suggest the presence of an evolutionarily conserved, sex-dependent dimorphism in responses to food scarcity

Changes in Physiology and Behavior

40% CR female rats

- ↑ activity
- ↓ BW
- ↑ corticosterone ~ hyperactivation of HPA axis ceased reproductive cycling
- ↓ gonadal size
- ↑ testosterone
- ↑ performance in cognitive tests

These changes would be expected to increase the probability of survival of the individual and species in a natural setting in which food is scarce
Females must obtain sufficient energy to support the survival and development of their offspring as well as themselves. Energy restriction appears to impose less stress and behavioral activation in males. The preservation of reproductive function and fertility in males during times of food scarcity. Allow to inseminate available fertile females. 

Johnson L et al. (1992)

Moderate energy restriction may decrease hunger in females. It is thought that the main function of leptin is to signal starvation when levels are low (conversely high levels signal the levels of body fat stores to the CNS in an environment of nutritional plenty). Increased adiponectin indicates increased insulin sensitivity.

Growth hormone

This study

Previous studies

• CR reduced GH in young male rats
• GH levels increased in 6- to 11-yr-old girls during a 48-h fast

Kasa-Wubu J et al. (2002)

Why GH levels decreased in response to CR in males but increased in females? unknown

But this sex difference could contribute to the differential effects of CR on male and female reproductive physiology. Jansson J et al. (1985), Jorgensen K et al. (1991)

Changes in Brain Neurotransmitters

Present study

DA levels in Hippocampus but not in other regions were reduced in response to CR and IF in females

Previous study

Changes in dopaminergic signaling in response to CR in rodents

Diao L et al. (1997), Carr K et al. (2003)

These findings suggest a possible role for changes in hippocampal dopaminergic signaling in behavioral and some hormonal responses of females & males to energy restriction. 

DA is known to play an important role in motivational and cognitive functions associated with hippocampus. D’Carroll C et al. (2006), Lemon R et al. (2006), Adcock R et al. (2006)

This study

• Hyperactivity and enhanced cognitive performance of female rats on the 40% CR
  - may involve alterations in dopaminergic signaling
  - another factor could be the differences in the states of food-entrainable oscillators compared to ad libitum-fed rats
CR Rats → Differences in the States of Food-Entrainable Oscillators

- Feeding schedules + CR → behavioral and physiological circadian rhythms and gene expression in the SCN are shifted and/or entrained.

Differences in the States of Food-Entrainable Oscillators

Previous studies

- Rats and mice can anticipate a fixed daily meal by entrainment of a circadian oscillator or clock.

- Scheduled food and/or water restriction → increases activity in the HP axis.
- Elevation in core body temperature around the time of feeding.

Female rats’ responses to caloric perturbation may shed light on human disorders of caloric intake

Previous studies

- AN is ~ 10 times more common in women compared with men.
- This sex difference has been suggested to be the result of the importance for women to maintain a slender and attractive body.
- Skrzypek S et al. (2001).

This study

- 40% CR female rats (assumedly free of body image issue) develop physiological and behavioral changes similar to those of women with AN.

Female rats’ responses to caloric perturbation may shed light on human disorders of caloric intake

AN-like physiological & behavioral alteration

- 20% CR
- Physical activity
- Reproductive shut down
- Hormonal masculinization
- Attention / Cognitive ability

Threshold level of energy restriction to which females respond by suppressing reproductive function and increasing motor and cognitive activities.

Conclusion
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- Female rats possess a greater sensitivity to perturbation of their caloric intake
- Female rats must obtain sufficient energy to support their survival and for nursing of offspring
- Endocrinological, neurophysiological and behavioral changes in females on food scarcity assist them in competing with males and other females for the remaining food in their environment
- A better understanding of these mechanisms may lead to novel strategies or therapies for the maintenance of a healthy body weight

Thank you for your attention